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THE EFFECTS OF THIOUREA AND PHENYLTHIOUREA UPON THE DEVELOPMENT OF ELEUTHERODACTYLUS RICORDII

W. GARDNER LYNN

Department of Biology, The Catholic University of America, Washington, D. C.

The leptodactylid toads of the genus *Eleutherodactylus* are unusual among anurans in that they lay large-yolked, unpigmented, terrestrial eggs and have no aquatic larval stage. The embryology of one member of this group, the Jamaican species *E. nubicola*, has been described in some detail (Lynn, 1942) and it was pointed out that during the development within the egg membranes certain characters which are found in ordinary aquatic tadpoles appear very transiently while others are never present at all. Thus, for example, the embryo possesses a broad vascular tail which is large and well developed during the latter part of intraoval life but degenerates and disappears near the time of hatching. Neither external nor internal gills are ever formed, there is no true opercular cavity and the ventral sucker and horny teeth of the typical tadpole are entirely lacking. The fore and hind limbs appear simultaneously and grow steadily throughout the embryonic period and at the time of hatching the young emerge as fully developed little frogs.

It is well known that in ordinary anurans such as *Rana pipiens* the metamorphosis of the tadpole into the frog is brought about through the agency of the thyroid gland which, late in larval life, begins a period of intense secretory activity resulting in a heightened level of thyroid hormone in the blood stream. The loss of gills and tail, shortening of the intestine, rapid growth and differentiation of the limbs, shedding of the chitinous beak, appearance of the tympanum and many other features of metamorphosis have been shown to be directly dependent upon this gradually increasing level of thyroid hormone. Thyroidectomized tadpoles remain in the larval state indefinitely unless thyroxin or iodine is administered to them. On the other hand, if normal tadpoles are treated with thyroxin at an early age they can be made to metamorphose precociously, producing tiny, but perfectly formed young frogs.

In view of these facts it seems possible that the telescoping of the larval stages in the development of *Eleutherodactylus* might be regarded as an exaggeratedly precocious metamorphosis resulting from an unusually early and intense functioning of the thyroid in this genus. The author has earlier presented some evidence, based on the histology of the thyroid at different embryonic stages, indicating that the gland is indeed precociously activated in this animal (Lynn, 1936). More recently Brink (1939) has made a study of the histology and cytology of the thyroid in *Arthroleptella bicolor villiersi*, a South African ranid with a somewhat similarly

abbreviated larval history, and has concluded that in this form also, the release of the thyroid hormone into the blood stream occurs at an unusually early stage.

It is clear that this evidence based upon the histological picture presented by the thyroid must be supplemented by experimental evidence before it can be regarded as conclusive. In the summer of 1941 the present author, working in Jamaica, B. W. I., attempted an experimental approach by removal of the pituitary anlage in the early embryo of *E. nubicola*. This operation, since it results in absence of the thyrotrophic hormone of the pituitary, prevents normal differentiation and functioning of the thyroid gland and thus provides a method for ascertaining the normal role of the thyroid in the developmental processes under investigation. Unfortunately these experiments were inconclusive because of the difficulty encountered in keeping the animals alive after the operation. Although the operation was relatively simple and involved removal of only a small bit of tissue, the embryos did not heal with the readiness exhibited by most amphibian larvae and despite repeated attempts, no operated animals survivied more than a few days.

With the recent development of various thyroid-inhibiting drugs, thiourea and related compounds (Kennedy, 1942; Richter and Clisby, 1942) and a number of the sulfonamides (Mackenzie, Mackenzie and McCollum, 1941; Mackenzie and Mackenzie, 1942, 1943; Astwood, 1943; Astwood, Bissell and Hughes, 1945), a new approach to the problem is possible since administration of these drugs will effectively block the production of thyroid hormone without the necessity of any surgical treatment.

The present paper is a report of such experiments carried out on the embryos of *Eleutherodactylus ricordii planirostris* (Cope), a species which is native to Cuba but has now, through accidental introductions, become well established in many parts of Florida.

Materials and Methods

The eggs used for this study were collected at Gainesville, Florida between August 19 and September 2, 1946.[1] The breeding habits of *E. ricordii* in Florida have been described by Deckert (1921), Skerner (1939) and Carr (1940). The eggs are laid in moist situations under loose boards or stones and the female remains with the eggs until they hatch. Goin (1947), in a study made at Gainesville, found that the average number of eggs per clutch is 16 and that the average period of development within the egg membranes is 15.6 days. For the present work 15 clutches of eggs were used. Each clutch was designated with a letter, the 15 groups thus constituting Series A to O. The total number of eggs available was 193 and the number per clutch ranged from 5 to 21.

Although these eggs are normally terrestrial, experience with the Jamaican species had shown that they can survive when immersed in water. It was therefore possible to administer the drugs simply by raising the eggs in the solutions, thus avoiding the necessity of injection. In each experiment a few eggs were kept on moist sand in a small flower-pot covered with a glass plate in order to simulate na-

[1] The author is indebted to Prof. J. Speed Rogers, Head of the Department of Biology, The University of Florida, for laboratory facilities during the course of the experiments and to Dr. C. J. Goin for suggestions and assistance in the collecting of specimens. Sectioning and study of the material was carried out at The Catholic University of America and at The Marine Biological Laboratory, Woods Hole, Massachusetts.

tural conditions; others were raised in tap-water and still others were raised in tap-water containing the appropriate concentration of the drug to be tested.

All of the eggs in a single batch are at the same stage of development at any given time but, of course, those of different batches were at various stages when collected. For this study only those series were used which were collected at the neural plate stage or earlier. All eggs were kept in the laboratory until they reached the early limb-bud stage before any treatment was instituted. This procedure was adopted because it insured that all embryos received treatment for comparable periods and also because previous experience had shown that successful removal of the jelly coats and vitelline membrane is difficult at earlier stages. At the limb-bud stage the thyroid gland is not yet differentiated (Lynn, 1936, 1942) so that in all of these experiments the treatment with thyroid-inhibiting drugs was instituted before the beginning of thyroid function.

Removal of the egg membranes was carried out in sterile Holtfreter's solution by means of finely ground watchmaker's forceps.

Daily observations were made under the binocular microscope and at various intervals embryos were fixed for sectioning. Fixation was in a 1:1 mixture of Bouin's fluid and cellosolve. The embryos were later dehydrated in cellosolve, cleared in xylol, sectioned at 10 μ and stained with Mallory's triple stain.

Results

1. Effect of the egg membranes upon the developmental rate of eggs raised in water.

Although eggs placed directly into tap-water were found to survive and to develop into froglets of normal appearance it became obvious early in the course of the work that such eggs were markedly retarded in their rate of development as compared with those kept in air on moist sand. On the other hand, if the jelly layers and vitelline membranes were removed from the eggs to be kept in fluid, then the developmental rate closely paralleled that of eggs kept on sand with all their coverings intact.

The details of a single experiment may be cited to illustrate this effect. The eggs of Series D were in early cleavage when collected on August 19. They were kept on moist sand for 5 days at which time all were well-developed embryos with both pairs of limbs present as buds, large vascular tails and lightly pigmented bodies (the "limb-bud stage"). At this time some of the eggs were dissected free of the surrounding jelly and membranes and placed in tap-water, others were placed in tap-water with all coverings intact and others were kept on moist sand. The eggs kept on sand developed normally and all hatched 10 days later (15th day of development). Eggs in tap-water without membranes paralleled those kept on sand. Differentiation and growth of limbs and digits, intensification of pigment, growth and later degeneration of the tail and other grossly visible changes occurred concomitantly in the two groups and at the time when the eggs kept in air were hatching, those kept in water were indistinguishable from them. Young frogs which were left in water after this time died within two days but those removed to moist sand lived normally. This is undoubtedly to be attributed to the change from cutaneous to pulmonary respiration which occurs at this time. During embryonic existence in the absence of any gills the respiration is cutaneous, probably mainly

through the thin-walled vascular tail. At late stages the tail begins to degenerate and it usually disappears within a day after hatching, when the lungs have come into function.

The eggs which were left in their membranes and raised in water showed a retardation almost immediately. Differentiation of the limbs was slow and the difference in pigmentation between these animals and the members of the control groups was particularly noticeable. By the 15th day of development, the time when the controls hatched, these embryos were at the stage which the controls had reached on the 8th day. They continued to develop slowly and reached what appeared to correspond to the hatching stage on about the 26th day. The embryos seemed weak however and none hatched spontaneously. When freed from the egg membranes by forceps they swam sluggishly, but when removed to moist sand they survived successfully.

The retarding effect of the egg membranes is shown in Figures 1 and 2 which are photographs of two individuals of Series H. These embryos were eleven days old when photographed and both had been kept in tap water from the 5th day of development. The embryo shown in Figure 2 had all coverings intact while that shown in Figure 1 had the membranes removed at the time of its immersion in water. The difference in the differentiation of the limbs and digits is particularly striking but sectioned material reveals that this is merely one aspect of a general retardation in developmental rate. It seems probable that this effect is due to a reduction of the rate of gas diffusion to and from the embryo, but no determinations of respiratory rate have been made to test this.

This effect having been demonstrated, all the experiments with thyroid-inhibiting drugs were carried out with eggs freed from the jelly layers and vitelline membrane.

2. Effects of thiourea treatment upon development.

A total of 71 eggs taken from 12 different batches was used for study of the effects of thiourea treatment. Three different concentrations were tested and, as previously noted, separate controls were run for each batch of eggs.

The lowest concentration of thiourea used was 0.001 per cent. Only 8 eggs, taken from Series A and B, were exposed to this concentration. The development of these did not seem to be affected in any way, the rates of growth and differentiation being the same as those of tap-water controls, and use of this concentration was therefore discontinued.

Twenty-four embryos from 5 different series of eggs were raised in 0.005 per cent thiourea. These animals showed no significant retardation in their rate of devel-

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PLATE I

FIGURE 1. Specimen from Series H, removed from jelly layers and vitelline membrane and placed in tap-water on the fifth day of development. Preserved and photographed on the eleventh day. × 8.

FIGURE 2. Specimen from Series H, raised in tap-water from the fifth to eleventh days with jelly layers and vitelline membrane intact. (Coverings removed before photographing.) × 8.

FIGURE 3. Specimen from Series H, raised in 0.005 per cent thiourea from the fifth to eleventh day of development. × 8.

FIGURE 4. Specimen from Series H, raised in 0.005 per cent phenylthiourea from the fifth to eleventh day of development. × 8.

PLATE I

opment but at the time when the tap-water controls were exhibiting marked degeneration of the tail, the tails of the experimental frogs remained large and vascular. This being the case, the latter animals were able to survive in water for an indefinite period while controls left in water invariably died when the tails had been reduced to small size. Because of the author's short stay in Florida, the longest period of survival of these experimental larvae beyond the time of death of the controls was 9 days. However, these animals were all active and healthy in appearance when fixed and there is no reason to suppose that they could not have lived much longer if some means of feeding them could have been found.

Figures 3 and 6 illustrate the effect of treatment with 0.005 per cent thiourea. The animal shown in Figure 3 is a specimen from Series H photographed at 11 days of age after 6 days in this solution. Comparison with the tap-water control of the same series (Fig. 1) shows that there is no significant difference between the two at this time ("pre-hatching stage"). Figure 6 shows an individual of Series N which was kept in 0.005 per cent thiourea for 11 days and a tap-water control of the same series is shown in Figure 5. It will be seen that the latter has passed the "hatching stage," having lost the tail and assumed the adult body form, while the experimental animal shows no signs of tail degeneration. Differentiation of the limbs in the two is, however, essentially the same.

Thirty-nine embryos from 7 different series of eggs were raised in 0.05 per cent thiourea solution. This concentration caused a definite retardation of development which first became apparent about 4 or 5 days after the beginning of treatment. From this time on, the experimental animals lagged behind the tap-water controls so that when the latter reached the "hatching stage," the former still had poorly developed digits, large vascular tails and ill-defined pigment patterns. Further development of these embryos was extremely slow and animals kept in 0.05 per cent thiourea for 10 days beyond the "hatching time" of the controls still exhibited several embryonic features in addition to the large larval tail. This is shown in Figures 7 and 8. These two embryos of Series C had been in fluid for 10 days. The control (Fig. 7) is very near the hatching stage with a much reduced tail and well-developed digits. The experimental animal (Fig. 8) has a tail of maximum size and shows considerable retardation of limb differentiation and pigment pattern development.

It is unfortunate that considerations of time made it impossible to carry these animals for longer periods beyond the "hatching time" for it appears probable that, in the case of embryos treated with 0.05 per cent thiourea, certain of the developmental features are not merely retarded but actually inhibted. Complete differentiation of the limbs was never attained and there was never any sign of metamorphic

PLATE II

FIGURE 5. Specimen from Series N, raised in tap-water from the fifth to the sixteenth day of development. × 8.

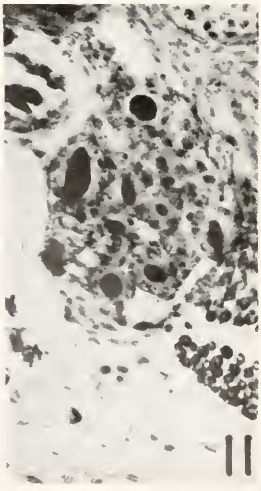
FIGURE 6. Specimen from Series N, raised in 0.005 per cent thiourea from the fifth to the sixteenth day of development. × 8.

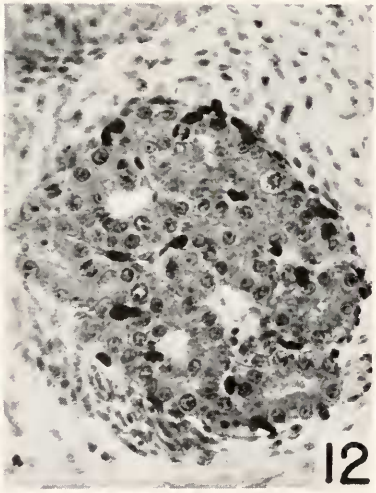
FIGURE 7. Specimen from Series C, raised in tap-water from the fifth to the fifteenth day of development. × 8.

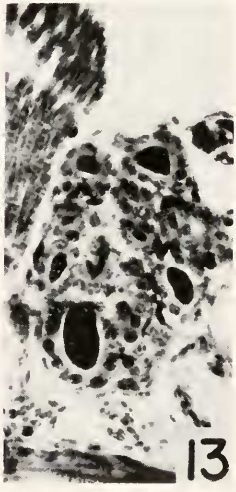
FIGURE 8. Specimen from Series C, raised in 0.05 per cent thiourea from the fifth to the fifteenth day of development. × 8.

PLATE II

PLATE III







degeneration of the tail during the course of the experiment. These are clearly effects which would be expected to result from inhibition of the metamorphosis-inducing properties of the thyroid but, since the animals when fixed still retained considerable amounts of yolk, the possibility remains that the effect is attributable to a general retardation of metabolic rate. Raising the embryos in 0.05 per cent thiourea for much longer periods, until complete utilization of the yolk, would doubtless settle this point.

3. Effects of phenylthiourea treatment upon development.

Twenty eggs from 5 different series were treated with 0.005 per cent phenylthiourea. These all showed a retardation of development which was essentially the same as that caused by 0.05 per cent thiourea. Degeneration of the tail was prevented and complete differentiation of the limbs and digits was never attained.

An additional and very striking effect produced by phenylthiourea, however, was a rapid and complete loss of pigment. At the limb-bud stage, when treatment was instituted, the embryos had a light peppering of melanophores over the dorsal surface and the pigmented coat of the retina was quite black. In every case, however, the experimental larvae were noticeably lighter than the controls by the third day after the beginning of treatment, and by the fifth or sixth day all visible dark pigment had disappeared. The eyes became white and the skin took on a translucent golden appearance indicating decoloration of the melanophores but not of the lipophores. It was also noted that these embryos after about ten days in the solution gave evidence of an abnormally high blood pressure or increased strength of heart-beat, the head and fore-limbs moving rhythmically with each heart-beat. The heart rate was not significantly different from that of the controls however.

An illustration of the effect of 0.005 per cent phenylthiourea upon general development and pigmentation will be seen in Figure 4. The animal shown in this photograph is another individual of Series H which was kept in the phenylthiourea solution for six days after the limb-bud stage. Comparison with the tap-water control (Fig. 1) or the animal kept in 0.005 per cent thiourea (Fig. 3) shows the pronounced depigmentation and the decided retardation in differentiation of the limbs. The loss of pigment in the pigmented coat of the eye is best seen in sectioned material as shown in Figures 9 and 10 which are photographs of sections of the eyes of the same animals shown in Figures 1 and 4. The pigmented coat and iris of the phenylthiourea-treated animal (Fig. 10) is almost completely decolorized despite the fact that this animal had been exposed to the drug for only 6 days.

4. Effects of thiourea and phenylthiourea upon the histology of the thyroid gland.

PLATE III

FIGURE 9. Section through the eye of the control animal shown in Figure 1. × 150.

FIGURE 10. Section through the eye of the phenylthiourea-treated animal shown in Figure 4. × 150.

FIGURE 11. Central section through the thyroid gland of the control animal shown in Figure 1. × 300.

FIGURE 12. Central section through the thyroid gland of the phenylthiourea-treated animal shown in Figure 4. × 300.

FIGURE 13. Central section through the thyroid gland of the thiourea-treated animal shown in Figure 3. × 300.

Examination of serial sections of control animals and those subjected to the various treatments described above provides a close correlation between the grossly visible effects upon development and the histological changes induced in the thyroid.

The results of treatment with 0.005 per cent phenylthiourea may conveniently be described first. Sections of the thyroids of experimental and control embryos fixed on the third day of treatment (eighth day of development) already exhibit well-marked differences. The control thyroid consists of relatively few small, primary follicles with low cuboidal epithelium and with the lumina occupied by a homogeneous blue-staining colloid. It represents a fairly early stage and only a mild degree of thyroid activity. The glands of the treated animal are only slightly enlarged but the follicular epithelium is predominantly columnar, some vacuolation of the colloid has occurred and the vascularization of the thyroid has increased. By the sixth day of treatment the contrast is much more striking and this stage has been chosen for illustration. Figure 11 is a photomicrograph of a central section of the thyroid of a tap-water control from Series H at this time. It will be noted that the follicular epithelium is cuboidal to low columnar and that all follicles contain fairly large masses of homogeneous red-staining colloid. This is a relatively active, but not a hyperactive, gland. The thyroid of the treated embryo is shown in Figure 12. It is markedly enlarged and the follicular epithelium is hyperplasic. Mitotic figures are common, three of them being seen in this photograph. Most follicles are completely collapsed and those which are not contain almost no stainable colloid. The hyperemia is indicated by the numerous blood corpuscles scattered about between the follicles. Essentially this same picture of intense activity is seen in all later stages studied which include animals up to the twenty-fourth day of development (nineteenth day of treatment) and the later stages are therefore not illustrated. There is no evidence in this material of any regression in activity during the period studied but perhaps this would have been observed if treatment could have been continued for a longer time.

The thyroids of animals treated with 0.05 per cent thiourea present the same picture as that produced by 0.005 per cent phenylthiourea and therefore need not be discussed in detail. It will be remembered that both of these treatments caused the same retardation in development.

Thyroids of animals kept in 0.005 per cent thiourea differ from those of controls only in that they are slightly enlarged and show increased vascularity. There is no significant difference in the amount or nature of the colloid present or in the height of the follicular epithelium. The thyroid shown in Figure 13 is that of the animal shown in Figure 3 which had been in 0.005 per cent thiourea for 6 days. It may be compared with the control of the same age (Figure 11). Animals treated for longer periods show no more pronounced effects. Despite the slight histological change exhibited by the thyroid in this case, some change in the amount or nature of the hormone produced must be postulated since tail degeneration is definitely prevented by this concentration.

The available sectioned material of animals treated with 0.001 per cent thiourea shows no points of difference from the controls. This was to have been expected since this concentration produced no detectible effects on development.

Discussion

Although only a few studies of the effects of thyroid-inhibiting drugs upon amphibians have as yet been made it has been conclusively demonstrated that these substances can produce effects upon the larva which are comparable to those resulting from thyroidectomy. Gordon, Goldsmith and Charipper (1943, 1945) showed that *Rana pipiens* tadpoles kept in 0.033 per cent thiourea retain the larval tail, gills and mouth-parts and fail to attain complete differentiation of the limbs. In other words they do not metamorphose, although they do continue to grow and may reach excessive sizes. Metamorphosis usually occurs promply when treatment ceases, although it may be delayed if treatment has been of long duration. Similar effects produced by thiouracil in a concentration of 1:2000 have been reported for *Rana clamitans* (Hughes and Astwood, 1944) and for *Rana pipiens* (Lynn and Sister Alfred de Marie, 1946).

On the basis of the present work it is clear that in *Eleutherodactylus*, as in *Rana*, there are certain developmental features which are dependent upon thyroid stimulation and are inhibited when normal production of the thyroid hormone is interfered with. The most noteworthy of these features are the resorption of the larval tail and the completion of differentiation of the limbs and digits. On the other hand, however, these experiments show that many of the developmental processes which are under thyroid control in ordinary anurans are to a greater or less degree, independent of such control in *Eleutherodactylus*. Thus even under conditions of what seems to be complete thyroid inhibition (treatment with 0.05 per cent thiourea or 0.005 per cent phenylthiourea) no tadpole-like mouth-parts, operculum or gills are ever formed and the limbs do develop to a considerable degree before showing any inhibition. In other words the treatment with thyroid-inhibitors does not result in the appearance of any larval characters which are normally absent in the species but it does cause an indefinitely prolonged retention of certain features which are normally very transient.

In an earlier discussion of this matter (Lynn, 1936) the author has pointed out that the evolutionary change which resulted in the atypical life history of *Eleutherodactylus* could conceivably have been brought about through a relatively simple genetic change, namely one which resulted in a precocious activation and functioning of the endocrine complex governing metamorphosis. The telescoping of the larval stages and early assumption of the adult body form could result from this and the later stages of intra-oval development could then be properly regarded as a precocious metamorphosis. The present experiments indicate quite clearly that the evolution of terrestrial development in these frogs can not be reduced to such simple terms. The thyroid stimulus undoubtedly plays a part in some of the later differentiations but many features of the embryogeny are carried out independently of the thyroid and it is obvious that in this anuran the genetic constitution is such that many of the tissues are able to undergo complete differentiation to the adult form without the endocrine intervention which is so essential in most amphibians. The development of the *Eleutherodactylus* embryo can, therefore, be more accurately described as a "direct development" rather than a "prococious metamorphosis within the egg."

The depigmentation effect exhibited by phenylthiourea but not by thiourea merits some discussion. Even before the discovery of the goiterogenic properites of

phenylthiourea it was reported by Richter and Clisby (1941) that continued administration of this substance to black rats causes graying of the hair, and also that cessation of treatment is followed by return of pigment. This phenomenon has been further studied by Dieke (1947) who finds also that alpha-naphthyl thiourea causes depigmentation of the skin of the rat. Neither of these drugs, in the doses used, had any effect upon the eye pigment of the rat.

Juhn (1944, 1946) has reported an effect of thiouracil administration upon feather pigmentation in the Brown Leghorn fowl but this seems to be attributable to the thyroid inhibition rather than to any direct influence upon the pigment cells for the modifications produced are the same as those which result from thyroidectomy.

Only one published account is concerned with the effects of thiourea derivatives upon the pigmentation of amphibians. This report (Lynn and Sister Alfred de Marie, 1946) records a reversible blanching observed in tadpoles of *Rana pipiens* raised in 0.05 per cent thiouracil. Further experiments in this laboratory have revealed that a definite depigmentation of the skin of *Rana pipiens* tadpoles is also produced by treatment with allylthiourea, phenylthiourea, amenobenzoic acid and sulfanilamide. In none of these cases, however, was the pigmentation of the eyes lost. The *Eleutherodactylus* embryo thus seems to be particularly susceptible to this action, showing much more complete and rapid depigmentation than does *Rana pipiens*.

It is noteworthy that a depigmentation of the skin and eyes very similar to that caused by phenylthiourea was reported much earlier (Lewis, 1932) in *Rana sylvatica* larvae treated with certain of the indophenol dyes. Moreover Figge (1938a), testing the effectiveness of these dyes upon various amphibian larvae, found a marked difference in sensitivity in different species. Larvae of *Necturus* were very readily depigmented in relatively low concentrations of the dyes; larvae of *Rana sylvatica* were somewhat less sensitive; larvae of *Amblystoma mexicanum* and *Rana catesbiana* were still less readily affected and *Amblystoma punctatum* larvae were least sensitive of all. Figge points out that this order of sensitivity parallels the order of metabolic rate of the different animals studied. *Necturus,* the most sensitive, has the lowest metabolic rate; *A. punctatum,* the least sensitive, has the highest; while *A. mexicanum* is intermediate in both respects. It is unfortunate that, because of the lack of any studies upon the metabolism of the *Eleutherodactylus* embryo, no conclusions concerning a possible relation between metabolic rate and sensitivity to the depigmentation effects of phenylthiourea can be drawn.

The basis for the depigmentation effect of the indophenol dyes has been investigated by Figge (1938b, 1939, 1940, 1941) who finds that phenol indophenol does not destroy pigment granules once formed but does prevent further formation of granules by affecting the enzyme system responsible for pigment production. Specifically, it was found that the dye inhibits the enzyme tyrosinase and thus prevents the production of melanin by the action of tyrosinase on tyrosine. The mechanism of the inhibition is apparently to be found in the fact that the dye shifts the oxidation-reduction potential of the substrate away from the optimum potential for tyrosinase activity. Presumably any substance which would cause such a shift in substrate potential would be equally effective in inhibiting pigment formation.

In view of these findings for the indophenol dyes, it is not surprising that recent studies indicate that the depigmentation effect of phenylthiourea and others of the thiourea derivatives are also to be attributed to tyrosinase inhibition although

whether the precise mechanism of inhibition is the same as that which seems to be obtained in the case of the dyes has not as yet been ascertained. Bernheim and Bernheim (1942) demonstrated inhibition of tyrosinase in vitro by phenylthiourea (phenylthiocarbamide), Paschkis, Cantarow, Hart and Rakoff (1944) have shown the effect for thiouracil, glutathione, cysteine, ascorbic acid, para-aminobenzoic acid, sodium sulfathiazole and sulfadiazine and Du Bois and Erway (1946) found that alpha-naphthyl thiourea is almost as effective as phenylthiourea in this respect while allyl-thiourea and thiourea are effective only in higher concentrations. The results of the present work are in agreement with this finding for, although 0.005 per cent phenylthiourea caused rapid and complete depigmentation of the embryo, thiourea, even at a concentration of 0.05 per cent, produced no discernible pigmentary changes.

It is of interest to note that in Figge's studies with the indophenol dyes these substances were found to produce, in addition to the pigmentary disturbances, specific effects on the eyes of some of the treated animals. The cells of the retina, lens and cornea were disoriented and the whole optic cup was collapsed and folded. No such effects have been observed in any of the animals subjected to the treatments employed in the present study. Even when the pigmented layer of the retina was completely lacking in melanin, the retinal layers themselves were normal in size and arrangement (Fig. 10). In this respect, therefore, the action of phenylthiourea differs from that of the indophenol dyes.

Summary

The leptodactylid toad, *Eleutherodactylus ricordii planirostris* (Cope), is unlike most anurans in that it possesses no aquatic larval stage. Its eggs are laid on land, beneath stones or logs. After about two weeks development within the egg, the young frogs hatch with the adult body form. It was found that eggs immersed in water will develop normally and at the usual rate providing the jelly layers and vitelline membranes are removed. In an attempt to ascertain to what degree the suppression of larval characters and the early assumption of the adult body form are dependent upon the activity of the thyroid gland, developing eggs were raised in solutions of thyroid-inhibiting drugs.

Embryos placed in 0.05 per cent thiourea or 0.005 per cent phenylthiourea on the fifth day of development failed to attain complete differentiation of the limbs and retained the larval tail, so that they were still embryonic in appearance 10 days after the tap-water controls had become complete little frogs. Animals raised in 0.005 per cent thiourea exhibited no retardation in limb development but did retain the larval tail so long as treatment was continued. Treatment with 0.001 per cent thiourea seemed to have no effect on development. Histological study of the thyroid glands of treated and control animals showed marked hyperplasia, hyperemia and reduction in colloid volume in the thyroids of specimens raised in 0.05 per cent thiourea or 0.005 per cent phenylthiourea. Thyroids of animals treated with 0.005 per cent thiourea showed slight hyperplasia and hyperemia but no significant differences in colloid volume as compared with controls. Thyroids of specimens raised in 0.001 per cent thiourea seemed to be unaffected. It appears that, in *Eleutherodactylus,* the loss of the larval tail and the complete differentiation of the limbs are features which are under thyroid control. On the other hand, the suppression or telescoping of many of the larval features cannot be attributed to thyroid activity since it occurs even under conditions of what seems to be extreme thyroid inhibition.

The embryos raised in 0.005 per cent phenylthiourea showed a rapid loss of pigment which involved not only the skin but also the pigmented coat of the eye. This is probably the result of the well-demonstrated inhibitory effect of this drug upon tyrosinase melanin formation.

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